Transient exposure of rat pups to hyperoxia at normobaric and hyperbaric pressures does not cause retinopathy of prematurity

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Abstract

We have shown that hyperoxia reduces brain damage in a rat model of hypoxia–ischemia. The purpose of this study was to examine the possibility of hyperoxia in inducing vision-threatening retinopathy. Two different experiments were conducted in this study. PART 1: seven-day-old rat pups were subjected to unilateral carotid artery ligation followed by 2 h of hypoxia (8% O2 at 37°C). Pups were treated with 100% oxygen at 1 ATA, 1.5 ATA, and 3.0 ATA for a duration of 1 h.
PART 2: Newborn rat pups were exposed to 100% oxygen at 1, 1.5, or 3.0 ATA for 1 h, the same treatment protocol used for brain protection after hypoxia–ischemia. Retinopathy was evaluated by the degree of neovascularization (measuring retinal vascular density), by the structural abnormalities (histology) in the retina, and by the expression of hypoxia–hyperoxia sensitive proteins including hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) at 24 h, 1, 2, and 10 weeks after hyperoxia exposure. Hyperoxic treatment at all pressures administered significantly reduced the hypoxia–ischemic-induced reduction in brain weight. Retinal vascular density measurements revealed no signs of neovascularization after hyperoxia exposure. There were also no abnormalities in the structure of the retina and no changes in the protein expression of HIF-1α and VEGF following hyperoxia exposure. Exposure to hyperoxia for 1 h at normobaric or hyperbaric pressures did not result in the structural changes or abnormal vascularization that is associated with retinopathy of prematurity, suggesting that hyperoxia is a safe treatment for hypoxic newborn infants.

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Introduction

The emergence of retinopathy of prematurity as a leading cause of blindness in infants has occurred over the past 60 years, seemingly as a result of the advances in neonatal intensive care practices that have allowed for the survival of premature infants who have significant immature retinal vasculature (Brooks et al., 2001; Miyamoto et al., 2002; Smith, 2002; Stout and Stout, 2003). The incidence for threshold (disease progression to the point of necessitating peripheral retinal ablation therapy) retinopathy of prematurity for premature infants weighing <1.25 kg is roughly 5%, with about 20–30% of these infants becoming blind despite treatment (Palmer, 2003; Reynolds, 2001).

Oxidant stress appears to play a role in the retinal vasoobliteration associated with retinopathy of prematurity (Beauchamp et al., 2002; Weinberger et al., 2002). Exposure to hyperoxia affects developing retinas by leading to microvascular degeneration which produces inner retinal hypoxia, which in turn leads to structural and functional changes. These changes can lead to abnormal vascularization resulting in the development of vision-threatening retinopathy (Beauchamp et al., 2002; Brooks et al., 2001). Hypoxia–ischemia is a common cause of brain injury in the perinatal period leading to mental impairment, seizures, and permanent motor deficits, such as cerebral palsy (Ferriero, 2001; Johnston, 2001). The role of supplemental oxygen therapy in the development of retinopathy of prematurity was suggested in the 1950s (Stout and Stout, 2003) and is a main reason for not giving oxygen to hypoxic or premature infants (Neubauer, 2002). But, recent data indicate that it is the withdrawal from the oxygen environment that causes retinopathy of prematurity and that subsequent oxygen exposure can cure...
the situation (The STOP-ROP Multicenter Study Group, 2000; Neubauer, 2002). We have shown that a single treatment of hyperbaric oxygen (HBO) (100% oxygen) for 1 h at 3 atmospheres absolute (ATA) attenuates brain damage caused by a hypoxia–ischemia insult on the neonatal rat brain by reducing the progression of apoptotic neuronal injury and increasing sensorimotor function (Calvert et al., 2002, 2003). Consideration of HBO treatment for hypoxia–ischemia is derived from the belief that oxygen under increased pressure might salvage the still viable, though nonfunctioning, tissue surrounding the area of insult by increasing the amount of oxygen dissolved in blood plasma, thereby increasing oxygen delivery to the brain (Nighoghossian and Trouillas, 1997). Also, by raising the amount of oxygen available to the brain tissue, hyperbaric oxygen may trigger a mechanism controlling cellular and vascular repair, presumably allowing time for tissue, hyperbaric oxygen may trigger a mechanism controlling cellular and vascular repair, presumably allowing time for collateral circulation to develop (Mink and Dutka, 1995). But, questions still remain about the safety of elevated levels of oxygen for the treatment of newborns, because experimentally, it has been shown that newborn rat pups exposed to oxygen at 1.8 ATA for 10 days did not show any signs of retinopathy of prematurity (Ricci and Calogero, 1988), whereas when newborn rat pups were exposed to a higher pressure of 5 ATA for 5 h, retinopathy of prematurity was evident (Torbati et al., 1995). Therefore, the purpose of this study was to test the hypothesis that a single exposure to 100% oxygen for 1 h at various pressures (1, 1.5, and 3.0 ATA) would not produce retinopathy of prematurity in newborn rat pups.

Materials and methods

Groups and oxygen exposure

The Animal and Ethics Review Committee at the Louisiana State University Health Sciences Center-Shreveport evaluated and approved the protocol used in this study. Timed pregnant female Sprague–Dawley rats were obtained from Harlan Labs (USA). After birth, pups were housed with the dam under a 12:12 h light/dark cycle, with food and water available ad libitum throughout the study. This study was divided into two parts.

Part 1: the model used in this study is based on the Rice–Vannucci (Rice et al., 1981) model previously described (Calvert et al., 2002, 2003). Unsexed 7-day-old (day 0 = day of birth) pups, Sprague–Dawley (Harlan) rats were anesthetized by inhalation with isoflurane (0.1%) in oxygen. The pups were kept at a temperature of 37 °C as the right common carotid artery of each pup was exposed and ligated with 5–0 surgical sutures. The duration of the anesthesia did not exceed 20 min, and the pups were allowed to recover with their dams for 2 h. They were then placed in a jar perfused with a humidified gas mixture (8% oxygen balanced nitrogen) for 2 h. Both the jar and the gas mixture were kept at 37 °C. The pups were returned to their dams after the hypoxic exposure. The pups that underwent hyperoxia treatment were allowed to recover from the hypoxic exposure for 1 h before being placed in the HBO chamber (Sechrist Industries, Inc., Anaheim, CA). The hyperoxic treatment of 100% oxygen was administered at pressures of 3 atmospheres absolute (ATA), 1.5 ATA, and 1.0 ATA for 1 h and the pups were then returned to their cages and dam after the treatment. Only one hyperoxic exposure was conducted for each pup. These pups were sacrificed 2 weeks after the hypoxic–ischemic insult. The pups in this part of the study were divided into the following groups: (1) control group (n = 5), (2) hypoxia–ischemia (HI) group (n = 15), (3). HI + 3.0 ATA group (n = 15), HI + 1.5 ATA group (n = 15), (4). HI + 1.0 ATA group (n = 15).

Part 2: unsexed 7-day-old (day 0 = day of birth) pups were divided into the following groups: (1) control group (n = 28), (2) normobaric group, 1.0 ATA group (n = 34), (3) 1.5 ATA group (n = 30), (4) 3.0 ATA group (n = 32), and (5) retinopathy of prematurity group (n = 12). Pups were placed in a hyperbaric oxygen chamber (Sechrist Industries, Inc) to expose them to oxygen. For 1, 1.5, and 3 ATA groups, oxygen (100%) was administered for duration of 1 h at the following pressures: 1 (normobaric), 1.5, and 3.0 ATA. Each pup only received one exposure. Control pups were not exposed to oxygen. The pups were sacrificed at various time points after hyperoxia exposure: 24 h, 1, 2, and 10 weeks. For the retinopathy of prematurity group, postnatal day 5 (P5) pups were exposed to 100% oxygen at a pressure of 3 ATA for 10 h. These pups were sacrificed at 24 h and 2 weeks to obtain positive controls for retinopathy.

Brain weight

The pups from Part 1 were sacrificed under deep anesthesia. After removal of the brain, the cerebellum and brain stem were removed from the forebrain. The hemispheres were separated by a midline incision and then weighed on a high precision balance (sensitivity ± 0.001 g). The cerebellum was also weighed. Brain damage was expressed as the percent reduction of the ipsilateral (right) hemisphere compared to the contralateral (left) hemisphere.

Retinal vascular density

Retinal vascular density was determined as previously described (Torbati et al., 1995). The thorax was opened and the left ventricle was perfused with 4 ml of India ink (Design Higgins, Sanford, USA) for pups in the 1 and 2 weeks groups and 12 ml for the rats in the 10-week group. After infusion of ink, eyes were enucleated and placed in 2% formalin. Under a dissecting microscope, the retinas were separated placed on glass microscope slides and mounted with coverslips. Images were captured using an imaging system that included an Olympus BX51 microscope with MagnaFire capturing software (SP2.1B) at magnifications of 1.25× and 10× and examined with Scion Image for windows (Scion Corp.). The retinal vascular density within an image was defined as...
the ratio of the total number of pixels representing the retinal vessels divided by the total number of pixels representing both the retinal tissue and retinal vessels.

**Histology and cell counts**

At the appropriate time (24 h, 2 and 10 weeks) after hyperoxia exposure, rats (n = 3 per group) were anesthetized with α-chloralose (40 mg/kg, i.p.)/urethane (400 mg/kg, i.p.). The thorax was opened and the left ventricle was perfused with 60 ml of 0.1M PBS (pH 7.4) followed by 60 ml of 2% glutaraldehyde and 2% formalin in 0.1M PBS (pH 7.4). Following perfusion, the eyes were enucleated and post-fixed in the same fixative and stored at 4°C. The eyes were cut across the conjunctiva and the cornea and crystalline lens were removed. The eye was then cut in half and placed in tissue freezing medium (Triangle Biomedical Sciences, USA). Four-micron sections were cut using a cryostat (Leica LM 3050S). Sections prepared from samples taken at 24 h after hyperoxia exposure were used for immunohistochemistry and sections prepared from samples taken at 2 and 10 weeks were stained with hematoxylin and eosin.

To analyze the retinal sections stained with hematoxylin and eosin, images of intact sections were captured at a magnification of 40× and analyzed using Image-Pro Plus 4.5.1 software. For each eye, two slides containing four sections were analyzed (representing whole eye). The number of neurons in the inner nuclear layer (INL), the number of neurons in the outer nuclear layer (ONL), the thickness of the outer plexiform layers (OPL), and the areas of both the INL and ONL were determined. For each section, these measurements were made in five distinct areas of the retina (see schematic in Fig. 4). The counts from each of these areas were then averaged to represent the counts for each section. The counts for each of these sections were then averaged to represent the counts for each eye.

**Immunohistochemistry**

Immunohistochemistry was done as previously described (Zhou et al., 2003) using an ABC Staining System (Santa Cruz Biotechnology, USA). Briefly, sections prepared from the samples taken at 24 h after hyperoxia exposure were used for immunohistochemistry. These sections were allowed to air dry before being washed in 0.01M PBS followed by incubation in 3% hydrogen peroxide (H₂O₂) to prevent reactions with endogenous peroxidases. This was followed by washing in PBS and then incubation with 3% normal serum in PBS (blocking solution). Sections were then incubated with either rabbit anti-VEGF antibodies (147) or rabbit anti-HIF-1α antibodies (H206) (Santa Cruz Biotechnology) diluted in blocking solution (1:100) overnight at 4°C followed by washing in PBS and incubation with anti-rabbit secondary antibodies. After washing again in PBS, sections were incubated with avidin–peroxidase complex solution containing avidin–peroxidase conjugate. The sections were then washed in PBS and peroxidase activity was revealed by dipping the sections in a mixture containing 3,3’-diaminobenzidine (DAB) and H₂O₂ followed by washing in distilled water. All the procedures were conducted at room temperature. Sections were allowed to air dry before being dehydrated and mounted with coverslips. Images of the slides were captured using the imaging system described above. Application of a control serum instead of the primary antibody on sections provided negative controls.

**Statistical analysis**

The retinal vascular density values as well as the values from the histological analysis are expressed as the means ± SEM. The values were analyzed by one-way analysis of variances (ANOVA), followed by Tukey test. A P value less than 5% was considered significant.

**Results**

**Brain weight**

To demonstrate that elevated levels of oxygen at normobaric and hyperbaric pressures can be neuroprotective, we subjected neonatal rats to a hypoxic–ischemic insult followed by 1 h of 100% oxygen at 1 ATA, 1.5 ATA, and 3 ATA. Two weeks after the insult and subsequent oxygen treatment, the pups were sacrificed and the brains of those pups were divided into two regions, contralateral hemisphere and ipsilateral hemisphere. Brain damage was then assessed by dividing the ipsilateral hemispheric weight by the contralateral hemispheric weight and expressing this as a percentage (Fig. 1). This rat model of hypoxia–ischemia yields a reproducible pattern of hemispheric injury ipsilateral, but not contralateral to the ligated carotid artery (Han et al., 2000). Since animals that are subjected to a hypoxic–
ischemic insult show retardation in brain growth, the percent reduction in ipsilateral brain weight to contralateral brain weight allows for the evaluation and testing of neuroprotective agents and strategies. The ipsilateral hemisphere was found to be 54.3% of the contralateral hemisphere after the hypoxic–ischemic insult and hyperoxic treatment at all pressures administered significantly reduced this damage, as brain weights were found to be 78.168%, 81.230%, and

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**Fig. 2.** Flat mounts of retinas of newborn rats exposed to hyperoxia at 1 atmosphere absolute (ATA), 1.5 ATA, or 3.0 ATA and age-matched controls. (A) One week after hyperoxia exposure. (B) Inversion of retinal images in A to provide a better look at the vasculature. (C) Two weeks after hyperoxia exposure. (D) Inversion of images in C. (E) Ten weeks after hyperoxia exposure. (F) Inversion of images in E. Scale bar = 1 mm.
70.497% after administration of 100% oxygen at 3.0 ATA, 1.5 ATA, and 1.0 ATA, respectively.

Retinal vascular density

We next tested if the hyperoxia that produced neuroprotection would result in retinopathy of prematurity. Retinal vasculature of newborn rat pups exposed to hyperoxia at various pressures (1, 1.5, and 3 ATA) is shown in Fig. 2. Higher magnification (10×) sections of the retinas (Fig. 3) were used in the calculation of retinal vascular density for each group (Table 1). No differences were observed in the retinal flat mounts at low magnification or high magnification and retinal vascular density calculations showed no difference among the control, 1.0, 1.5, and 3.0 ATA groups at 1 (data not shown), 2, and 10 weeks after hyperoxia exposure.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>INL (no. of cells)</th>
<th>INL no./area (no./μm²)</th>
<th>OPL thickness (μm)</th>
<th>ONL (no. of cells)</th>
<th>ONL no./area (no./μm²)</th>
<th>RVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 2 weeks</td>
<td>118 ± 7.077</td>
<td>0.0127 ± 0.000583</td>
<td>16.527 ± 0.115</td>
<td>317 ± 0.391</td>
<td>0.0229 ± 0.000930</td>
<td>0.0421 ± 0.00596</td>
</tr>
<tr>
<td>1 ATA 2 weeks</td>
<td>112 ± 3.228</td>
<td>0.0136 ± 0.000322</td>
<td>16.882 ± 0.286</td>
<td>326 ± 9.426</td>
<td>0.0258 ± 0.000521</td>
<td>0.0463 ± 0.00252</td>
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<tr>
<td>1.5 ATA 2 weeks</td>
<td>123 ± 1.350</td>
<td>0.0144 ± 0.000512</td>
<td>16.387 ± 0.187</td>
<td>331 ± 8.161</td>
<td>0.0259 ± 0.000589</td>
<td>0.0383 ± 0.00374</td>
</tr>
<tr>
<td>3.0 ATA 2 weeks</td>
<td>117 ± 1.802</td>
<td>0.0133 ± 0.000577</td>
<td>16.759 ± 0.168</td>
<td>325 ± 6.423</td>
<td>0.0238 ± 0.000146</td>
<td>0.0416 ± 0.00410</td>
</tr>
<tr>
<td>ROP 2 weeks</td>
<td>106 ± 1.951</td>
<td>0.0166 ± 0.00147</td>
<td>9.150 ± 0.453*</td>
<td>334 ± 6.001</td>
<td>0.0281 ± 0.000119</td>
<td>0.110 ± 0.00667*</td>
</tr>
<tr>
<td>Control 10 weeks</td>
<td>116 ± 4.655</td>
<td>0.0130 ± 0.000429</td>
<td>16.068 ± 0.381</td>
<td>336 ± 9.346</td>
<td>0.0241 ± 0.000103</td>
<td>0.0457 ± 0.00304</td>
</tr>
<tr>
<td>1 ATA 10 weeks</td>
<td>121 ± 1.562</td>
<td>0.0131 ± 0.000715</td>
<td>16.718 ± 0.232</td>
<td>335 ± 6.624</td>
<td>0.0242 ± 0.000147</td>
<td>0.0444 ± 0.0163</td>
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<tr>
<td>1.5 ATA 10 weeks</td>
<td>121 ± 1.280</td>
<td>0.0134 ± 0.000121</td>
<td>16.195 ± 0.345</td>
<td>336 ± 4.809</td>
<td>0.0246 ± 0.000568</td>
<td>0.0442 ± 0.00460</td>
</tr>
<tr>
<td>3.0 ATA 10 weeks</td>
<td>122 ± 1.822</td>
<td>0.0124 ± 0.000269</td>
<td>16.777 ± 0.410</td>
<td>332 ± 2.517</td>
<td>0.0242 ± 0.000328</td>
<td>0.0436 ± 0.00524</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SEM. INL indicates inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RVD, retinal vascular density. *P < 0.001 (ANOVA) compared to all other groups in 2-week time point.
exposure. We did expose some pups to 100% oxygen at 3 ATA for 10 h to induce retinopathy. Newborn rat pups in this retinopathy of prematurity group did show signs of increased vascular proliferation in their retinas 2 weeks after hyperoxia exposure when compared to age-matched controls (Fig. 4). There was also a significant increase ($P < 0.001$, ANOVA) in retinal vascular density values for the retinopathy of prematurity group at 2 weeks compared to the other groups at the same time point (Table 1).

Histological analysis

The effects of hyperoxia on the structure of the retina were further assessed on serial sections of the retina (Fig. 5). The following parameters were measured: the number of cells in the inner nuclear layer (INL) and outer nuclear layer (ONL), the thickness of the outer plexiform layer (OPL) ($\mu$m), and the area of both the INL and ONL ($\mu$m$^2$) (Table 1). The number of cells per unit area was also determined. No difference was found in the number of cells in the INL, the thickness of the OPL, and the number of cells in the ONL among the control, 1, 1.5, and 3.0 ATA groups at both 2 and 10 weeks. Retinas of newborn pups in the retinopathy of prematurity group (Fig. 4) had an OPL that had a thickness of $9.150\pm0.453$, which was significantly ($P < 0.001$, ANOVA) thinner (arrow Fig. 4) than the OPL of the age-matched control group as well as the 1, 1.5, and 3.0 ATA groups at 2 weeks after hyperoxia exposure. There were no differences in any of the other parameters measured.
HIF-1α and VEGF protein expression

HIF-1α and VEGF protein expressions were assessed in the retinas of newborn rat pups 24 h after hyperoxia exposure. HIF-1α protein expression (Fig. 6) was evident in retinas of pups in all groups examined. Labeling was observed at the level of the inner retinal surface near the nerve fiber layer and within the INL. No differences in HIF-1α protein expression were noted between the control, 1, 1.5, and 3.0 ATA groups. However, HIF-1α protein expression pattern was different in the retinas of retinopathy of prematurity pups. The majority of HIF-1α expression was found to be in the inner limiting membrane (Fig. 6E, arrow), which is the layer closest to the retinal vessels and there was also less expression evident in the inner retinal surface near the nerve fiber layer and within the INL. Labeling of VEGF (Fig. 7) was also observed at the level of the inner retinal surface near the nerve fiber layer (NFL) and within the INL. No differences in the extent of expression of VEGF were noted between the control group, 1, 1.5, and 3.0 ATA groups. VEGF protein expression in the retinopathy of prematurity group, like the HIF-1α protein expression, was also found to be in the inner limiting membrane with more intense

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Fig. 6. HIF-1α protein expression 24 h after hyperoxia exposure. (A) Age-matched control, arrow denotes positive HIF-1α staining. (B) 1 ATA group. (C) 1.5 ATA group. (D) 3.0 ATA group. (E) retinopathy of prematurity group, arrow denotes HIF-1α expression in the inner limiting membrane. 100×, Scale bar = 10 μm.

Fig. 5. Hematoxylin and eosin staining of retinas from newborn rat pups 2 and 10 weeks after exposure to hyperoxia. (A–B) Age-matched controls. (C) 1 ATA group, 2 weeks. (D) 1 ATA group, 10 weeks. (E) 1.5 ATA group, 2 weeks. (F) 1.5 ATA group, 10 weeks. (G) 3.0 ATA group, 2 weeks. (H) 3.0 ATA, 10 weeks. (I) retinopathy of prematurity group, 2 weeks. Note the thinning of the OPL in the retina from the retinopathy of prematurity group (I, arrow). Abbreviations in panel A: INL, inner nuclear layer; ONL, outer nuclear layer; OPL, outer plexiform layer. Schematic in right lower corner shows regions analyzed for each section. Scale bar = 20 μm.
staining seen (Fig. 7E, arrow) and there was less expression evident in the inner retinal surface near the NFL and within the INL.

**Discussion**

The retina appears to only be susceptible to oxidant stress during developmental stages because maturational events occurring around the 36th postconceptual week in humans and the third postnatal week in rodents make the vessels resistant to hyperoxia-induced obliteration (Brooks et al., 2001). If the use of supplemental oxygen therapy (pressurized or not) is to be used in the treatment of premature or hypoxic infants, questions concerning the safety of hyperoxia, especially in regards to the retina, need to be addressed. We show here, that hyperoxia at pressures ranging from 1.0 ATA to 3 ATA is neuroprotective when administered after a hypoxic–ischemic insult and that hyperoxia, as used in this study, does not cause the structural changes or abnormal vascularization in the retina that are associated with retinopathy of prematurity. We also found that there are not any differences in the expression of HIF-1α or VEGF in the retinas of pups exposed to hyperoxia when compared to control pups. Thus, a single 1-h application of oxygen, either normobaric or hyperbaric, appears to be a safe treatment protocol for neonates after a hypoxia–ischemia insult.

Hypoxic conditions can trigger neovascular proliferation through the induction of potent angiogenic factors such as VEGF (Ricci et al., 2000), which is regulated by HIF-1α under hypoxic conditions (Miyamoto et al., 2002). During a hypoxic–ischemic insult, the HIF-1α-VEGF system is thought to provide protective measures by reconstructing the vasculature through the development of collateral vessels, thus acting as a rescue mechanism. However, this does not appear to be the case in the eye (Miyamoto et al., 2002). The pathophysiology of retinopathy of prematurity can be
separated into two distinct phases. Phase I is termed hyperoxia-vasoconstriction and occurs when an infant or animal is placed in a hyperoxic environment. The hyperoxia can cause a downregulation of VEGF leading to the cessation of normal retinal vascularization (Reynolds, 2001; Shih et al., 2003). Phase II is termed hypoxia-vasoproliferation and occurs when the infant or animal is moved from the hyperoxic environment back to room air causing a relative hypoxia to develop in the retina. An increase in VEGF in response to this hypoxia can lead to the proliferation of vessels that can invade the vitreous and the retina appearing as the pathological phenomena that cause the various clinical and experimental stages of retinopathy of prematurity (Provis et al., 1997; Stone et al., 1995). Subjecting mice to 75% oxygen from postnatal day (P) 7-P12 leads to the development of extraretinal neovascularization by P17 (Brooks et al., 2001) through an increase in VEGF protein expression when the mice are returned to room air (Brooks et al., 2001; Pierce et al., 1996). Our results showed that a single exposure to hyperoxia did not result in an over-expression of VEGF when the pups were returned to room air for 24 h, nor did we see any indications of an increase in retinal vascularization within 10 weeks after the exposure. Torbati et al. (1995) have shown that retinopathy of prematurity can develop in newborn rats exposed to 100% oxygen at 5 ATA for 5 h, whereas Ricci and Calogero (1988) have shown that exposing newborn rats to 80% oxygen at 1.8 ATA for 10 days after birth did not cause retinopathy of prematurity. However, Ricci and Calogero (1988) also showed that exposing newborn rat pups to 80% oxygen at normobaric pressure for 5 or 10 days after birth did cause retinopathy of prematurity. The vasoconstrictive response caused by hyperbarism can be used to explain the results of both studies. Hyperbarism can constrict the choriocapillaries and reduce the amount of oxygen transported from the choroid to the inner retina during the period of hyperoxia. The vasoconstrictive response will vary with the degree of hyperbarism, so at high pressures the constriction can cause a severe and prolonged reduction in choroidal and retinal blood flow. On return to room air after exposure to low levels of hyperbarism, the hypoxic environment the retina finds itself in will be reduced and the stimulus for vasoproliferation will be decreased (Ricci and Calogero, 1988). On return to room air from exposure to higher levels of hyperbarism, oxidative damage created by hypoxia–ischemia can lead to the induction of retinal vasoproliferation (Torbati et al., 1995). These results and those of this study suggest that it is the duration of the exposure that is important when hyperoxia is given at normobaric pressure, that the pressure is important when hyperoxia is given at hyperbaric pressures, and that hyperoxic exposures can be safe when administered at the appropriate pressure and duration.

Exposure to hyperoxia during the first 14 days of postnatal life in rat pups can prevent the normal development of the OPL and result in long-lasting effects that can cause the structural anomalies in the retina that are associated with prolonged hyperoxia exposure.
oxia–hypoxia in very low birthweight infants (Chow et al., 2003). In light of the potential toxic effects and caution surrounding the use of elevated levels of oxygen at normobaric pressures, treatment with oxygen at increased pressure may be a more suitable treatment for premature and hypoxic infants. Mild hyperbarism, as mention above, has been shown to be safe, in regards to the retina, when administered to rats for 10 days (Ricci and Calogero, 1988) and HBO is being used to treat newborns and children with radiation-induced bone and soft tissue complications, cyanotic congenital heart disease, and CO poisoning (Ashallalla et al., 1996; Chuba et al., 1997; Rudge, 1993). As mentioned above, consideration of HBO treatment for hypoxia–ischemia is derived from the belief that HBO therapy might salvage the still viable, though non-functioning tissue surrounding the area of insult through an increase in the amount of oxygen available to the brain tissue (Nighoghossian and Trouillas, 1997). Other possible effects of HBO include the reduction of pressure within the brain caused by swelling, the restoration of blood–brain barrier function and cell membrane function, the scavenging of free radicals, and the reduction in the stickiness of blood products (Neubauer, 2002). All of which aid in the reduction of brain injury. In recent years, HBO treatment has emerged as a treatment for cerebral palsy (CP), a common pathology associated with a hypoxia–ischemic insult during the perinatal period. Cases studies have shown that administration of HBO (sometimes years after the initial injury) to children who had experienced perinatal hypoxia–ischemia led to the dramatic improvement of these patients in regards to behavior and alertness (Neubauer, 2002).

It needs to be pointed out that the purpose of this study was not to induce retinopathy of prematurity in neonatal rats (hence, we did not use the established model of oxygen-induced retinopathy), only to test if a single treatment of hyperoxia at various pressures would result in the retinal changes associated with retinopathy. In conclusion, we have shown that a single exposure to 100% oxygen for 1 h at normobaric or hyperbaric pressures did not cause retinopathy in newborn rat pups, suggesting that when used correctly hyperoxia does not lead to the development of retinopathy of prematurity. The results obtained in this study may help to open the door for the use of hyperoxia therapy for the treatment of hypoxic newborn infants since both normobaric oxygen (Singhal et al., 2002a,b) and hyperbaric oxygen (Badr et al., 2001; Yin et al., 2002) have been shown to be effective in neonate and adult models of stroke.

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References


